Author's response to reviews

Title: Study protocol of the B-CAST study: a multicenter, prospective cohort study investigating the tumor biomarkers in adjuvant chemotherapy for stage III colon cancer

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Author's response to reviews: see over
Dear Professor Patel,

Thank you for reviewing our manuscript entitled “Study protocol of the B-CAST study: a multicenter, prospective cohort study investigating the tumor biomarkers in adjuvant chemotherapy for stage III colon cancer” (MS: 2087622143784951) and giving valuable suggestions.

According to the referee’s comments, we prepared the revised manuscript and response to referees. Please find enclosed the revised manuscript, and take into consideration for publication, again.

Below are response to referee and a summary of the changes.

**Comment from referee #1:**

1) The details on chemotherapy using a web-based program. It is unclear regarding how much information/accuracy of exact chemotherapy regimens, doses and AE will be adequately reported.

    > According to your suggestion, we added a list of collected information regarding adjuvant chemotherapy (table 2).

2) Due to the number of biomarkers being tested, note should be made in statistical analysis regarding correction for multiple testing.

    > Since this is an observational study (not an experimental study), we consider that adjustment for multiplicity is not necessary. We added a
following sentence into page 21:
“Since this is an observational study (not an experimental study), no adjustment for multiplicity is applied.”

Comment from referee #2:
1) It would be good if the authors can describe how the patients were staged clinically – that is, by CT scans, endoscopic ultrasound, colonoscopy and so on. Presumably TNM staging is used; if so, it would be useful to state that as well.

> The study protocol does not define how the patients are staged clinically. So we do not describe this in the manuscript. Practically, colonoscopy, barium enema examination and CT are used routinely in Japan, but EUS is rarely used for ≥cT3 tumors.
And as you pointed, the 7th UICC-TNM staging system is used in the study. We stated it in the revised manuscript (page 11).

2) Clinical and pathological features need to be collected. The paper only mentions pathological features. A list of all data collected would be useful.

> Thank you for your suggestion. According to your suggestion, we added a list of collected demographic and pathological data (table 1).

3) After the pathology results confirmed that the patients have stage III colon cancer with clear margins, the patients are offered adjuvant chemotherapy. The tumor specimens of the patients who did not go on have adjuvant chemotherapy, were not analyzed and discarded. Would it be useful to have a control group who did not receive adjuvant chemotherapy or are the numbers too small to be use? It would be useful to know the DFS, RFS and OS of this particular group who did not have chemotherapy.

> As you supposed, the number of stage III CRC patients who did not receive adjuvant chemotherapy is small to be analyzed. And they often have factors
which correlate to poor health status and poor prognosis (i.e. older age, severe comorbidities). So we considered that the DFS, RFS and OS of these patients are not appropriate for comparison with those of patients with adjuvant chemotherapy.

4) How would the correlation between each biomarker and RFS/DFS/OS be performed and would the differences between the different biomarkers be analyzed in patients with or without recurrence? Kindly elaborate.

> In “ANALYSIS PLAN” section, there was misleading information in the first sentence that “correlated” is ambiguous and not proper. In this study, the Cox proportional hazard model with covariate as tumor biomarker expression is performed to evaluate significant relationship with DFS/RFS/OS in each treatment group. And as as exploratory analyses, univariate and multivariate relationships among survivals, expression of biomarkers and patient characteristics will be evaluated using the Cox proportional hazard model.
We revised some sentences in “ANALYSIS PLAN” section (page 20-21).

5) With regards to adhering to the CONSORT methods of reporting, the authors might considering changing the below mentioned:

After “Final enrolment” on page 14, there could be a subheading for “Treatment” and description of adjuvant therapy administered and follow up schedule (currently termed “outcome survey” on page 16).

> According to your suggestion, we created a “TREATMENT” section and described about adjuvant chemotherapy and surveillance in the section (page 16-17).

On page 16, instead of the subheading “collecting information of adjuvant chemotherapy”, there could be a subheading under “outcome”. This would also include definition of endpoints (currently under “statistical matters”)
and the paragraph outlining the collection of AEs.

> According to your suggestion, we created a “TREATMENT OUTCOME” section and described definition of clinical endpoints (efficacy and safety) in the section (page 17-18).

6) The writing needs to be improved as there are grammatical mistakes and certain sentences are ambiguous.
Some examples:

Page 9 – thymidine phosphorylase (TP), which is more in tumor tissues than normal tissues, with the aim of reducing gastrointestinal toxicity and hematotoxicity of 5-FU. – It might read better if changed to: ....thymidine phosphorylase (TP), which is in higher concentrations in tumor tissues than in normal tissues, with the aim of reducing gastrointestinal and hematological toxicities of 5-FU.

> Thank you for your kind advice. We revised the sentence as you suggested (page 9).

Page 16 – The study protocol dose not defines treatment regimens and schedule for assessment of AEs. After chemotherapy is completed, the following information is reported using a Web-based case report system. – So there is a schedule for reporting on the web?

> We revised the sentences as follows (page 16-17):
“The study protocol does not define treatment regimens and schedule of hospital visits during chemotherapy. When the chemotherapy is finished, the following information regarding adjuvant chemotherapy is reported using a Web-based case report system: treatment regimen, dose of each drug, ....”

Page 19 - *: According to the accumulation situation of the case, the registration period was extended from 2 years to 3 years on February 2011.
> We revised the sentences as follows (page 20):
“*: Because of the delayed cumulative pace of patient enrollment, the registration period was extended in February 2011, from 2 years to 3 years.”

**Comment from referee #3:**

1) Since this paper reports study protocol, and no results, further detail on methods is appropriate.
The methods state that a tissue block was taken from the primary tumor immediately after surgery. Was this by the surgeon or the pathologist at cut up?

> In Japan, most surgical specimens are prepared for histopathological examination by surgeons. The protocol does not define who cut tissue blocks. We suppose that the majority (probably >95%) of tumor blocks in the present study are cut by the surgeons.

Were there any guidelines as to which part of the primary tumor should be sampled?

> In this study, tissue blocks are ordered to be taken from “the land wall” of the primary tumor. In the revised manuscript, we described this point clearly in “COLLECTION/STORAGE/SUBMISSION OF TUMOR SAMPLES” section (page 13).

More details would be helpful as this block was wholly digested for DNA/RNA analysis.

> As you mentioned, the tissue blocks are “wholly” digested for ELISA/RNA analyses. In the revised manuscript, we described this point clearly in “MEASUREMENT OF BIOMARKERS” section (page 15).
Was there any QA eg on adjacent blocks, to see if the majority of the tissue frozen was tumor?

> No, we do not. However, we consider that the most part of the specimen taken from the land wall consist of tumor tissues.

Was there a subset in which two blocks were taken from the primary tumor to verify similar results?

> No, we do not have any subset for verifying the similarity of biomarker expressions between two blocks.

2) Should be an explanation of why/why not there is no influence on the biomarkers of oxaliplatin in combination v those treated with FU derivatives alone.

> Patients receiving oxaliplatin containing regimens will be evaluated separately from those with 5-FU derivatives alone. We described it in the revised manuscript (page 20).

3) Is there a standard protocol for post-adjuvant treatment monitoring? – This may affect DFS as the timing of reporting of asymptomatic relapse relates to the frequency of investigations, hence this should be specified in the protocol.

> We quite agree with you. As we mentioned in “SURVEILLANCE FOR RELAPSE” section (page 17), in this study, surveillance after adjuvant chemotherapy is recommended to be performed in accordance with the JSCCR guidelines. In the revised manuscript, we added a figure of the recommended schedule of surveillance (Figure 3).
4) More details on the analysis plan once all the biomarkers have been evaluated is needed. Will there be a multivariate analysis?
> Please see the response to comment from referee #2, 4).

The protocol dose not report collection of basic demographic data eg age, comorbidities which influences DFS.
> Please see the response to comment from referee #2, 2).

We are looking forward to hearing from you.

With best regards,

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